N E W S L E T T E R

CLOVERS AND SPECIAL PURPOSE LEGUMES RESEARCH

Vol. 5--1971

Compiled by the Forage and Range Research Branch
Plant Science Research Division, Agricultural Research Service
U.S. Department of Agriculture, Beltsville, Maryland

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INTRODUCTION

The objective of this Newsletter—Clovers and Special Purpose Legumes Research—is the informal exchange of research information on these many species. The contents of each volume include voluntary contributions compiled without editing. We encourage the future use of this medium for the exchange of research information not available by other media.

We hope the Newsletter will serve to disseminate current research information such as that given at research conferences. As you know, we have but few research conferences concerning the many species involved. Perhaps we should give serious consideration to a Clovers and Special Purpose Legumes Research Conference early in this decade of the 1970's. Advise me of your thoughts on this matter.

We welcome contributions and suggestions in future issues of the Newsletter. In short, this Newsletter is what you make it.

Contributions to the Sixth Issue of the "Newsletter—Clovers and Special Purpose Legumes Research" may be sent to:

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Thank you,
Bob Leffel

ALABAMA

Sericea Lespedeza, Vetch, and Arrowleaf Clover

Carl S. Hoveland and E. D. Donnelly (Auburn)

Sericea lespedeza (Lespedeza cuneata). Interstate, a new variety developed primarily for roadside vegetation, was released and seed are being increased commercially. Irradiated seed resulted in a mutant from which Interstate was developed. The new sericea is shorter growing, branches more profusely, has finer stems, and grows more uniformly than other varieties tested in Alabama. Interstate also appears to have promise as a grazing and hay crop.

Forage yields of Abruzzi rye, rescuegrass, and Gulf ryegrass seeded annually for three years on Serala sericea were compared. Rye furnished 1,400 kg/ha of dry forage by March 20 while rescue and ryegrass produced less than 250 kg/ha by the same date. Rye extended the productive season by about 2 months and increased total annual yields 2,000 kg/ha over the 6,600 kg/ha produced by sericea alone. Sericea growth in spring was not reduced by rye, but it was reduced by rescue and ryegrass. None of the species reduced the stands of sericea.

Vetch (Vicia). Nova, a reseeding vetch variety, was released for use as a winter grazing or green manure crop. Nova vetch will reseed in clean cultivated summer crops if allowed to produce seed at least once every 2 or 3 years. Crops such as soybeans or grain sorghum can be grown after Nova vetch has matured seed. Sclerotinia trifoliorum will limit the potential use of Nova vetch. This is a much more severe problem on old established bermuda and bahia sod than on prepared land.

Four advanced-generation, hard-seeded vetch lines (Vicia sativa type) from V. sativa L. X V. cordata Wulf. were studied for persistence of the hard seed characteristic in subsequent generations. Lines differed in persistence of hard seed. Two lines produced approximately 75% hard seed over a 3-year period whether planted from successive generations of hard seed only or from hard and soft seed as produced. Two other lines that received these same treatments averaged over the 3-year period 26 and 30% less hard seed when hard and soft seed were planted each generation than when hard seed only were planted. Data indicated that two lines were homozygous for the gene or genes conditioning hard seed and that two lines were segregating.

Arrowleaf clover (Trifolium vesiculosum). Poor recovery growth after cutting in spring has been a problem with Yuchi arrowleaf clover. Two factors thought to affect regrowth were studied in field experiments at two locations for 2 years. Total available carbohydrates in roots were not related to regrowth after cutting. Number of buds at the base of the clover plants were closely related to regrowth. Bud numbers remained high until early April under all cutting frequencies. After early April, bud numbers at base of the plants remained high under

frequent cutting (simulated grazing) but declined sharply when clover was cut less frequently. Cutting at hay stage in late April or early May resulted in no regrowth of clover. Maximum yields of forage (over 3 1/2 tons per acre) from this winter annual clover were obtained by frequent cutting (or grazing) until early April followed by a cutting of hay in late May.

Arrowleaf clover forage harvested from a management experiment for 2 years was found to be high in digestible dry matter (DDM). DDM values ranged from 90% in winter to 70% at early bloom hay stage in late May. Tannin content of forage ranged from 3 to 6% and was not affected by season. Leaves were higher in tannin than stems. The relatively high tannin content of the forage, possibly acting as an anti-foaming agent, may explain the absence of bloat in cattle grazing this clover. Amino acid analyses showed the forage to be high in lysine, suggesting possible use for swine and poultry feed. Zinc content was also very high.

In a 3-year field study at two locations, various herbicides were applied before planting Yuchi arrowleaf clover. DCPA was a satisfactory herbicide and the clover tolerated rates as high as 12 lb/acre. Forage yields of clover were not reduced at the first or second harvest when compared to an untreated control. Little barley control was excellent. Benefin and chloropropham at low rates were generally satisfactory but injury might occur if long drought periods occur during germination.

In a 3-year grazing trial on Abruzzi rye-Gulf ryegrass-Yuchi arrowleaf clover, yearling steers grazed continuously from November or early December until June with no supplements. Daily gains per steer averaged 2.07 pounds. The average gain per steer was 400 pounds. Clover supplied most of the grazing in April and May. Stocking rate was one steer per acre until spring when additional animals were used. Carcasses of steers slaughtered directly off pasture graded USDA Good or better. Objectionable fat pigmentation was not observed. Pasture cost per pound of gain was 12¢ as compared to 20¢ with steers receiving corn silage in feedlot.

Publications:

1. Anthony, W. B., C. S. Hoveland, E. L. Mayton, and H. E. Burgess. 1971. Rye-ryegrass-Yuchi arrowleaf clover for production of slaughter cattle. Auburn Univ. (Ala.) Agr. Exp. Sta. Circ. 182.

2. Buchanan, G. A., and C. S. Hoveland. 1971. Tolerance of Yuchi arrowleaf clover to herbicides. Weed Sci. 19:254-256.

3. Donnelly, E. D. 1970. Persistence of hard seed in Vicia lines derived from interspecific hybridization. Crop Sci. 10:661-662.

4. Donnelly, E. D., and W. B. Anthony. 1970. Lowering tannin content improves quality of sericea lespedeza forage. Auburn Univ. Agr. Exp. Sta. Highlights of Agr. Res. Vol. 17, No. 1.

5. Donnelly, E. D., R. Dickens, D. G. Sturkie, and J. D. Miller. 1970. Interstate sericea lespedeza--new variety for a special purpose. Auburn Univ. Agr. Exp. Sta. Highlights of Agr. Res. Vol. 17, No. 2.

6. Hoveland, C. S., and E. L. Carden. 1971. Overseeding winter annual grasses in sericea lespedeza. Agron. J. 63:333-334.

7. Hoveland, C. S., G. A. Buchanan, and E. D. Donnelly. 1971. Establishment of sericea lespedeza. Weed Sci. 19:21-24.

8. King, C. C., and J. W. Langford. 1970. Nova vetch--new variety for row crop rotations. Auburn Univ. Agr. Exp. Sta. Highlights of Agr. Res. Vol. 17, No. 3.

9. Robinette, D. L., D. W. Speake, and E. D. Donnelly. 1968. An evaluation of a reseeding vetch, clanton tick clover, and a low-tannin selection of sericea lespedeza as a quail food and cover plants. Proc. 22nd An. Conf. Southeastern Assoc. Game and Fish Commissioners; pp. 1-12. (Paper not previously cited in Legume Newsletter.)

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CANADA

Self-fertility in Species of Sweetclover (Melilotus)

A. T. H. Gross and G. A. Stevenson
(Brandon, Manitoba)

Plants of nine annual and nine biennial species were grown in the greenhouse and their self-fertility was determined on unmanipulated and manipulated florets. More than 300 florets of each annual species and more than 1000 of each biennial were examined for seed set in each category.

Four plants of each accession were transplanted to the greenhouse in the first week of November and flowering commenced in mid-January. Four pots with four or more seedlings of each annual accession were started in mid-October and flowering commenced in mid-January. A daylength of 14 hours was maintained by use of fluorescent lights. Temperature and humidity conditions were standardized for the period of test.

Table 1. Percent seed set of 9 annual and 9 biennial *Melilotus* spp., Brandon, Manitoba

Annuals *Melilotus* Species	Seed Set % unmanipulated	Seed Set % manipulated	Biennials *Melilotus* Species	Seed Set % unmanipulated	Seed Set % manipulated
elegans	79.1	86.9	alba	4.7	52.3
indica	95.9	--	altissima	4.5	82.5
infesta	63.2	--	dentata	86.1	94.4
italica	71.7	87.4	hirsuta	11.0	42.3
macrocarpa	49.4	69.7	officinalis	0.4	26.0
messanensis	70.3	80.6	polonica	8.7	60.9
neapolitana	95.0	98.3	suaveolens	1.3	38.4
officinalis[1]	1.0	80.1	taurica	6.9	42.1
speciosa	19.7	55.2	wolgica	33.5	46.4
sulcata spp. brachystachys	93.2	--	early[2]	1.6	25.1
sulcata spp. segetalis	22.4	51.3	late	5.7	59.4
Average	55.0	74.8		21.3	54.7

[1] an annual form of the biennial species.

[2] January florets (early), March florets (late).

The annuals tended to be more self-fertile than the biennials. Spontaneous self-fertility was consistently lower than self-fertility of manipulated florets. Late florets were more self-fertile than early florets. The comparative self-fertility within annuals and within biennials were similar for both spontaneous (unmanipulated) and manipulated conditions.

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The Potential for Crownvetch in Ontario

Edited by E. M. Watkin, D. Bielby, and J. E. Winch (Guelph, Ontario)

Proceedings of symposium held at the University of Guelph
January 1971; 107 pp. *

I The need for ground cover crops in Ontario
II Characteristics of crownvetch
III Current experience, future problems in crownvetch use

CONTENTS

* Copies of the symposium are available from E. M. Watkin, University of Guelph, Guelph, Department of Crop Science, Ontario, Canada.

Crop protection chemicals for crownvetch seed production
R. A. Woods, Chipman Chemicals Ltd., Hamilton

Legal aspects of crop introduction
W. E. Sieveking, Maple Leaf Mills, Georgetown

DISCUSSION

D. Bielby, Ontario Department of Agriculture and Food

SUMMARY - J. E. Winch, Department of Crop Science, University of Guelph

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COLORADO

Effect of Seed Size and Seeding Depth on Seedling Emergence of Two Milkvetch Species

C. E. Townsend (Ft. Collins)

Introduction: Seedling vigor is a character of special significance for crops seeded under dryland conditions. In some crops there tends to be a relationship between seed size and seedling vigor with the larger seeds producing the more vigorous seedlings. In conjunction with seed size, seeding depth has been suggested as a method of selecting for seedling vigor. It is generally believed that larger seeds will emerge from greater depth than will smaller sized seeds; hence the greater vigor of seedlings from the larger seeds.

Astragalus cicer and A. falcatus are two species which we believe may have a place in the dryland agriculture of this region. Both are long lived perennials with A. cicer spreading by rhizomes and A. falcatus having a tap root similar to alfalfa. We are evaluating the suggestion that larger seeds may produce more vigorous seedlings.

Methods: Bulked seed lots of the two species were sized by screens into different sizes - three for falcatus and four for cicer. All seed was scarified and inoculated with Rhizobium prior to planting. Seed was planted at three depths - 1/2 inch, 1 inch, and 1 1/2 inches - at the rate of 1 seed per inch of row. The experimental design was a split plot with four replications. Whole plots were depth of seeding with subplots consisting of seed size. A subplot was a row 20 feet in length with 12 inch spacing between rows. Seedings were made in April or May and the stand counts were made in June by counting the number of seedlings in 10 feet of row.

Results: A. cicer - In 1969 there was no difference between the 1/2 inch and the 1 inch seeding depths for seedling emergence but these depths gave significantly better emergence than did the 1 1/2 inch depth (Table 1). There was also considerable difference among seed sizes for seedling emergence. The three largest seed sizes gave better seedling emergence than did the small class. Although size 12 (largest) did not differ significantly from size 14, the difference approached significance. Also, seedling emergence for size 12 seed was better than for other seed sizes at all seeding depths. These data indicate that larger seeds tend to give better seedling emergence than do small seeds.

In 1970 significant differences were found among seeding depths for seedling emergence (Table 2). It was surprising that the best emergence was from the 1 1/2 inch depth, although it was only slightly better than for the 1 inch depth. Both the 1 inch and 1 1/2 inch depths were considerably better than the 1/2 inch depth. This is in contrast to the 1969 data where the 1/2 and 1 inch depths gave much better emergence than did the 1 1/2 inch depth. Seasonal differences for seedling emergence are attributed to differences in soil moisture conditions at seeding time and to subsequent precipitation. In 1969 the soil was moist at seeding time and no additional precipitation was received by the time the seedling counts were taken. In 1970, the soil surface was very dry at seeding, but there was some moisture at the lower depths. Additional precipitation was not received until a few days before the stand counts were made. Significant differences occurred among seed sizes for seedling emergence. As expected, the small class gave the poorest emergence. The best emergence was obtained from size 13 seed, but size 12 seed (largest) did not differ significantly from sizes 13 and 14.

A. falcatus - The first year of this study was 1970. Significant differences were found among seeding depths for emergence (Table 3). As with A. cicer, emergence from the 1/2 inch depth was less than from the 1 inch and 1 1/2 inch depths. No difference was found among seed sizes for seedling emergence.

Conclusions: The 1 inch seeding depth is the best overall depth for seeding A, cicer and A. falcatus. There appears to be a relationship between seed size and seedling vigor in A. cicer with the larger sized seeds giving the best emergence. Thus far, we have not found a similar relationship in A. falcatus.

Table 1. Number of seedlings per foot from different seed sizes of A. cicer which emerged from three seeding depths. Ft. Collins, 1969.

Seeding depth	Seed Size				
	Small	14	13	12	Avg.
1/2 inch	1.0	1.9	3.0	3.3	2.3
1 inch	0.6	3.3	2.9	3.9	2.7
1 1/2 inches	<0.1	0.7	0.8	0.9	0.6
Avg.	0.5	1.9	2.3	2.7	
L.S.D. 5% for two seeding depth means = 1.2					
L.S.D. 1% for two seed size means = 0.9					

Table 2. Number of seedlings per foot from different seed sizes of A. cicer which emerged from three seeding depths. Ft. Collins, 1970

	Seed Size				
Seeding depth	Small	14	13	12	Avg.
1/2 inch	0.3	0.7	1.3	1.8	1.1
1 inch	1.1	2.7	3.3	3.1	2.6
1 1/2 inches	1.6	2.8	4.0	3.0	2.9
Avg.	1.0	2.1	2.9	2.6	

L.S.D. 1% for two seeding depth means = 0.6
L.S.D. 1% for two seed size means = 1.0

Table 3. Number of seedlings per foot from different seed sizes of A. falcatus which emerged when seeded at three depths. Ft. Collins, 1970.

	Seed Sizes			
Seeding depth	14	13	12	Avg.
1/2 inch	2.7	2.4	2.9	2.7
1 inch	4.6	4.3	5.2	4.7
1 1/2 inches	3.8	4.3	4.0	4.0
Avg.	3.7	3.7	4.0	

L.S.D. 1% for two seeding depth means = 1.1

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FLORIDA

Evaluation of Clovers and Frost Lupine

Leonard S. Dunavin (Jay)

Variety trials. -- Oven-dry forage yields of white clovers, crimson clovers, arrowleaf clovers, and Frost lupine are presented in Table 1 from variety trials conducted in 1970-71. These trials were planted on October 27, 1970, on a Red Bay fine sandy loam soil. Preplanting fertilization consisted of 500 lbs. per acre of 0-14-14. Rainfall was generally adequate but irrigation was used on May 7 and June 4, 1971. Five replications were used for the white clovers and four for the others.

Grazing trials. -- Total beef gains per acre from trials utilizing Dixie crimson clover, Amclo arrowleaf clover, and Frost lupine were: rye, ryegrass, and crimson 539 lbs.; rye, ryegrass, and arrowleaf 535 lbs.; wheat, ryegrass, and crimson 521 lbs.; wheat, ryegrass, and arrowleaf 497 lbs.; and lupine 374 lbs. Daily gains were best on lupine but the period of grazing was too short.

Table 1. Oven-dry forage produced by several clovers and Frost lupine. Agricultural Research Center, Jay, Florida, 1970-71.

Variety	Pounds Per Acre				
	16 Mar	13 Apr	5 May	8 June	Total
Amclo arrowleaf	1434	1368	3234	599	6635
Yuchi arrowleaf	1016	1350	3242	772	6380
Meechee arrowleaf	348	1190	3751	915	6204
Chief crimson	2069	1900	2635	0	6604
Common Oregon crimson	2160	1640	1937	0	5737
Early Reseeding crimson	2558	794	2076	0	5428
Autauga crimson	2456	1131	1749	0	5336
Auburn crimson	2609	745	1699	0	5053
Frontier crimson	2595	591	1829	0	5015
Frost lupine	2981	290	692	0	3963
White clovers:					
Tillman	271	915	1831	1709	4726
Nolin's Improved	375	959	2197	865	4396
Ladino	306	780	1579	1531	4196
Merit	81	607	1768	1653	4109
Regal	281	934	1751	1026	3992
Espanso	194	671	1630	1488	3983
Ladino Gigante	153	710	1501	1422	3786
WFES #1	196	802	1958	707	3663
La. S-1	218	732	1799	836	3585
Minn. A Ladino	0	460	1823	790	3073

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IDAHO

Domestic Exploration for Native Lupines

A. E. Slinkard (Moscow)

In 1968, a domestic collection of native lupines was made in eastern Washington, eastern Oregon, Idaho and adjacent Montana. In 1969, 69 accessions were established in the field. in 1970, data were recorded on flower color, seed yield and alkaloid content. A summary of the data is available on request. Accessions were given P.I. numbers and seed was supplied to the Western Regional Plant Introduction Station, Pullman, Washington.

Fourteen accessions were tentatively identified as being low in alkaloids. In 1971, attempts will be made to intercross several low alkaloid accessions.

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NEW JERSEY

The Effect of TIBA on Crownvetch Seed Production

(Excerpt from Report to
International Minerals & Chemical Corporation)
F. B. Gaffney (Cape May)

Crownvetch is an indeterminate bloomer and thus is a problem to harvest partially because it sets seed over a long period of time and partially because second growth starts before the seed crop is mature. An evaluation of TIBA was undertaken by the Cape May Plant Materials Center in hope that it would cause crownvetch to bloom and set one seed crop and possibly reduce regrowth.

During June of 1970, the following procedure was followed:

Three rates, 10, 20, and 30 grams per acre of TIBA (IMC-3889)-plus a surfactant; applied three times at two week intervals; replicated three times; and were sprayed on 1/200 acre plots on 'Chemung' crownvetch.

The weather from July 4, until after crownvetch harvesting was extremely dry. No irrigation water was applied until after seed harvest.

It was originally intended to take individual plot seed weights. However, no observable differences were noted in seed set, so plots were not harvested separately. Very little difference in regrowth was noted. The untreated rows had slightly less regrowth on them; however, no quantitative data was taken.

After two years of trials no promising results have occurred. More complete details are available upon request.

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NORTH CAROLINA

North Carolina Report for 1971

Will A. Cope (Raleigh)

Publications:

1. Cope, W. A., and J. O. Rawlings. 1970. Inheritance of forage yield and certain morphological and fruiting characteristics in crownvetch. Crop Sci. 10:550-553.

2. Cope, W. A., and Joseph C. Burns. 1971. Relationship between tannin levels and nutritive value of sericea. Crop Sci. 11:231-233.

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PENNSYLVANIA

Self-seed Production of Red Clover

W. A. Kendall (University Park)

Self-seed Production of Red Clover: This study was initiated at the Kentucky Agricultural Experiment Station and transferred to the U.S. Regional Pasture Research Laboratory during July 1970. Objectives of the present research are to improve the selfing technique previously described (Theoretical and Applied Genetics 39: 1969) to enable greater and more consistent seed production, and to gain further knowledge of the mechanism of self-incompatibility.

A new incubator was developed to control temperature, humidity, and air circulation around the flower head during anthesis. The instrument consisted essentially of a box about 15 inches square with about 12 holes of 1 inch diameter placed at random over two sides and the bottom of the box. Flower stems were oriented through the holes so that flower buds at the distal end of the stems were inside the incubator while the basal end of the stems were either left intact on a potted plant or were inserted in a jar of nutrient solution as in the earlier techniques. The unit was heated by a 200-watt strip heater which was thermostatically controlled. Humidity could be regulated only above the ambient by exposing pans of water with various surface areas inside the incubator. Air circulation was provided by a 3-inch fan mounted in the top of the box with the air stream directed over the heater strip and toward the bottom of the box.

In the new incubator the nutrient solution is maintained at the ambient room air temperature and the need for a refrigerated water circulation system is eliminated. The new unit also makes possible the treatment of flower heads on intact stems. Generally seed production has been greater from flowers on intact stems than from flowers on excised stems. Relatively low amounts of air movement and low relative humidity (less than 50%) have favored seed production.

Self-seed set on excised stems has not been improved by supplementing the media with antibiotics (malathione, omadine, chloramphenicol, sodium floride, tannin, streptomycin sulfate, sulfanilamide, iodoacetic acid, and N^6 benzyladenine); inorganic nutrients (calcium chloride, calcium nitrate); plant hormones (gibberellic acid, kinetin, indole acetic acid and indole butyric acid). In some experiments the number of seed obtained was increased with proline in the media at about 1,500 ppm.

Growth of Pasture Species

A study of the effects of genotype, environment and certain root pathogens on growth rates of shoots and roots of various pasture plant species has been initiated. Several cultivars and ecotypes of red clover trefoil and crownvetch will be included.

Publications

1. Newton, Darwin L., W. A. Kendall, and N. L. Taylor. 1970. Hybridization of some Trifolium species through stylar temperature treatments. Theoret. Appl. Genet. 40:59-62.

2. Kendall, W. A., R. H. Lowe, and N. L. Taylor. 1971. Growth of red clover pollen. III. Free amino acid composition in grains and supplements to culture media. Crop Sci. 11:112-114.

3. Taylor, N. L., C. J. Keller, M. K. Anderson, and W. A. Kendall. 1971. Anthocyanidin floral pigmentation in Trifolium pratense L. J. Hered. 62:13-15.

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Red Clover - Root Borer

K. T. Leath and R. A. Byers (University Park)

The ability of adult clover root borers (Hylastinus obscurus) to select between healthy and diseased roots was investigated using a walking bioassay technique. Borers collected from infected roots from the field were caged with access to water leachates from healthy and diseased root pieces. More than 80% of the borers in several tests preferred the leachate from diseased roots.

In three tests, borers placed on pieces of healthy roots walked to and bored into pieces of diseased roots contained within the same petri dish. No borers left pieces of diseased roots in preference for healthy roots.

Borers did not seem to be attracted to leachates from fungi or bacteria isolated from diseased roots and cultured on potato-dextrose-agar. Borers were attracted to roots naturally infected in the field with several organisms and also to roots artificially inoculated with Fusarium roseum or Colletotrichum trifolii.

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SOUTH CAROLINA

Trifolium Investigations

O. W. Barnett, Chi-Chang Chen, and Pryce B. Gibson (Clemson)

1. Trifolium repens L. germplasm tolerant to root-knot incited by Meloidogyne incognita (Kofoid & White) Chitwood -- During the period 1959-1971, 145 elite white clover clones have been identified by screening plants from various sources for tolerance to root-knot. In April 1971, 10 plants of each clone were shipped to Prosser, Washington. In cooperation with the Seed Production Investigations Section, PSRD, ARS, seed will be produced from this material. The seed Mr. C. M. Rincker harvests will be available to plant breeders.

2. Summary of Interspecific Hybridization Studies -- The following chart summarizes our results to date.

Crosses and Results

	1 T.r.	2 T.n.	3 T.p.	4 T.m.	5 T.o
1. T. repens L. (T.r.)					
2. T. nigrescens Viv. (T.n.)*	S**				
3. T. petrisavii Clem. (T.p.)	S	S			
4. T. meneghinianum Clem. (T.m.)	S	S	S		
5. T. occidentale D. E. Coombe (T.o.)	S	S	S	F	
6. T. uniflorum L. (T.u.)	S	F	F	F	S

*According to Hossain 2, 3, and 4 comprise one species, nigrescens, which he divided into subspecies nigrescens and subspecies petrisavii. He divides petrisavii into two varieties, petrisavii and meneghinianum.

**Letters at the intersection of two species indicate the results we have obtained by crossing the two species. S=successful, viable plants obtained. F=failure.

All of these hybrids have been reported in published papers or in manuscripts now being considered for publication with the exception of the hybrid between T. repens and T. meneghinianum. If. T. meneghianum is classified as a subgroup of T. nigrescens, success in obtaining this hybrid merely extends success in hybridizing T. repens and T. nigrescens to the third subgroup of T. nigrescens.

3. Search for a 2n= 16 T. repens -- We screened several thousand white clover seedlings for twins. Our objective was to find a haploid plant as has been done in some species. We found one seedling with twin primary stems. Neither had a chromosome complement of 2n=16.

   We have just begun to explore the possibility of obtaining a 2n=16 plant, by culturing immature anthers.

4. Karyotypes of 15 Trifolium species in Section Amoria. -- The karyotypes of 15 species of Trifolium belonging to Section Amoria Presl. were compared on the bases of chromosome size, centromere position, number of satellite chromosomes, and size of satellites. Some species have similar or indistinguishable karyotypes while others differ from one another by one or more cytological characters. The similarity of karyotypes of T. nigrescens, T. occidentale, T. petrisavii, and T. repens suggests a close phylogenetic relationship among these species.

5. Cytogenetic relationships among several Trifolium species. -- Chromosome association in interspecific hybrids was used to determine the relationships among several species of Trifolium. The materials used and the results obtained are summarized in Table 1. From this table the following conclusions may be drawn. (1) There is considerable homology among the gametic chromosome complement of T. repens, indicating a possible autotetraploid origin of this species. (2) T. uniflorum is a natural autotetraploid. (3) There is considerable homology among the chromosomes of T. nigrescens, T. occidentale, and T. repens, indicating a close phylogenetic relationship among these species. (4) There is homology between some of the chromosomes of T. uniflorum and those of T. occidentale and T. repens.

6. Seed development following the mating of Trifolium repens x. T. uniflorum. -- About 17% of the ovules of T. repens were fertilized by the male gametes of T. uniflorum following the mating of the two species, however, few, if any, of these would have developed into viable seeds. The hybrid embryo, although it grew more slowly than that of T. repens, differentiated normally or nearly so up to the sixth day after pollination. The hybrid endosperm, on the other hand, began to develop abnormally on the fourth day. By the sixth day, the endosperm was highly vacuolate. The appearance of the endosperm and the adjacent maternal tissues suggested that at this stage of development the endosperm had lost the ability to absorb and conduct nutrients. By the eighth day, the endosperm had disintegrated and the embryo appeared to be degenerating. These observations suggest that failure of the hubrid embryo to develop to a germinable condition following the mating of T. repens x T. uniflorum is a result of starvation caused by abnormal development and, eventually, disintegration of the endosperm. Failure of the endosperm to nourish the embryo may be attributed to genetic imbalance of the endosperm itself, or to an unfavorable interaction of the endosperm with the maternal tissues.

7. Viruses of white clover. -- Since the last newsletter report, more Trifolium species in the section Amoria have been tested for susceptibility to clover yellow vein (CYVV), bean yellow mosaic (BYMV), red clover vein mosaic, clover yellow mosaic, white clover mosaic, alfalfa mosaic, and peanut stunt viruses. T. ambiguum shows promise

Table 1. Chromosome associations in *Trifolium* species and hybrids

Species or hybrid	Chrom. number (2n)	Cells observed	Chromosome association at MI							
			IV		III		II		I	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
T. repens	32	34	0.03	0-1	0.00	-	15.88	14-16	0.12	0-2
T. nigrescens	16	41	0.00	-	0.00	-	8.00	-	0.00	-
T. occidentale (2x)	16	76	0.00	-	0.00	-	7.90	7-8	0.19	0-2
T. occidentale (4x)	32	33	4.64	1-8	0.12	0-2	6.82	0-12	0.42	0-4
T. uniflorum	32	93	4.43	2-7	0.02	0-1	7.10	2-12	0.02	0-1
T. nigrescens x *T. occidentale* (4x)	24	99	0.00	-	5.69	2-8	2.31	0-6	2.31	0-6
T. nigrescens x *T. repens*	24	133	0.00	-	4.27	0-8	3.73	0-8	3.73	0-8
T. repens x *T. meneghinianum*	24	25	0.00	-	3.16	1-5	4.80	3-7	4.92	3-7
T. repens x *T. occidentale* (2x)	24	29	0.00	-	4.55	2-8	3.45	0-6	3.45	0-6
T. repens x *T. occidentale* (4x)	32	26	4.50	1-8	0.27	0-2	6.12	0-12	0.88	0-4
T. uniflorum x *T. occidentale* (4x)	32	34	2.81	0-6	0.08	0-1	9.69	4-16	0.38	0-2
T. repens x *T. uniflorum*	32	160	1.24	0-4	0.54	0-3	10.83	4-16	4.24	0-16
T. repens x [*T. unif.* x *T. occid.* (4x)]	32	107	2.49	0-6	0.67	0-3	9.10	3-16	1.95	0-10
[*T. repens* x *T. occid.* (4x)] x *T. uniflorum*	32	54	2.04	0-4	0.04	0-1	11.41	7-16	0.85	0-4

as a source of resistance to all seven viruses. More than 50% of the white clover breeding clones maintained at Clemson were shown to be infected with CYVV with an antiserum conjugated with latex particles. CYVV infects both white and red clover but no white clover has been infected with either of two strains of BYMV by mechanical inoculation. Serological and infectivity assays of CYVV under field conditions revealed a relatively high virus concentration throughout the winter. A graft-transmissable enation disease of clover was found in the greenhouse. Symptoms of this disease include enations and line patterns on the upper leaf surface of white clover, T. occidentale, or interspecific crosses between the two. On T. occidentale dwarfing and proliferation may accompany the enations.

Publications:

1. Chen, Chi-Chang and Pryce B. Gibson, 1970. Chromosome pairing in two interspecific hybrids of Trifolium. Canadian Journal of Genetics and Cytology 12:790-794.

2. Gibson, Pryce B. and Chi-Chang Chen, 1971. Reproduction and Cytology of Trifolium uniflorum. Crop Science 11:69-70.

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TEXAS

Legume Species Evaluation at Beaumont

G. W. Evers*, A. W. Smith and J. P. Craigmiles (Beaumont)

Climatic conditions of the upper Texas Gulf Coast are ideal for lush clover growth. Clover is so abundant that it frequently causes bloat in cattle--especially during early spring where phosphate fertilizers have been applied. White clover is the predominant species with La S-1 being the leading variety. Many clovers and legumes show promise on these low, damp soils. In the past, selection and breeding programs at the Beaumont Center have included T. resupinatum, T. nigrescens, T. michelianum, T. alexandrinum, and Melilotus alba var. annua.

To evaluate various forage legumes, a test was established on Morey silt loam October 6, 1969, at Beaumont. Twenty entries were seeded in small plots. Plant emergence was poor in some species and harvest was not warranted. Prior to planting, seeds of each legume were inoculated with the required Rhizobium Spp. and 150 lbs. of 0-24-24 fertilizer was applied to the area. The seed and incoulum were provided by R. C. Leffel.

*Dr. Gerald W. Evers joined the Beaumont Center staff Dec. 1, 1970. He obtained his B.S., M.S., and Ph.D. degrees from Texas A&M University. His main area of interest is in forage physiology and management. He will be leader of the forage research at Beaumont, which includes legume research.

One clipping was made April 29, 1970, and the following results obtained:

Entry	Pounds Dry Forage/Acre
Birdsfoot Trefoil (Lotus corniculatus)	
Leo	2032
Empire	2739
White Clover (T. repens)	
Regal	3830
Ladino	2940
Merit	2767
La S-1	3565
Abon Persian Clover (T. resupinatum)	3746
Ball Clover (T. nigrescens)	5569
Mike Clover (T. michelianum)	5383
Berseem Clover (T. alexandrinum)	4009
Onar (Onobrychis viciaefolia)	3020

Lotus corniculatus var Dawn and Viking; Lotus pedunculatus; Trifolium ambiguum var. Kura; Coronilla varia var. Penngift and Emerald; Astragalus cicer var Eski; and Corniculatus (Md 1) had poor seedling emergence and failed to develop good stands. Consequently, yield determinations were not made.

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WISCONSIN

Red Clover Breeding and Genetics

R. R. Smith and D. P. Maxwell (Madison)

Screening red clover populations and individual plants for resistance to viruses (Wis. pea streak virus, WPSV; red clover vein mosaic virus, RCVMV; bean yellow mosaic virus, BYMV; and alfalfa mosaic virus, AMV), rust, northern anthracnose, and powdery mildew were continued. No resistance to WPSV was detected in over 2,000 plants tested. Four isolates of AMV are being used to determine if more than one gene in red clover is involved in resistance to the different AMV isolates. Clones of red clover which are resistant to RCVMV have been obtained and are being used in inheritance studies. Serological procedures are being developed for the rapid estimation and identification of legume viruses. Clones resistant to rust were obtained from two red clover introductions (P.I. 210370 and 304784). Further genetic investigations are being

conducted. After four cycles of mass selection for resistance to BYMV, 75 percent of the plants in the population are resistant as compared to 38 percent for Lakeland.

Screening for resistance to northern anthracnose in red clover was performed in the greenhouse over a three-year period. Eleven breeding lines were crossed separately with a red clover clone resistant to northern anthracnose. Progeny from these crosses were inoculated and surviving plants within each line were intercrossed. The subsequent progeny was inoculated and 22 resistant clones were intercrossed. When tested for resistance, the progeny of the 22-clone polycross showed a ten-fold greater survival rate (50 percent resistant) than Lakeland. This screening work is being continued. Inheritance studies on northern anthracnose resistance suggest that more than one gene controls the resistant condition.

Plants in Lakeland yield plots established in 1968, 1969, and 1970 (3rd year, 2nd year, and 1st year stands) were examined for the presence of virus and clover root borer (Hylastinus abscurus) prior to the first harvest in 1971. In addition, plants of Wis. Syn H yield plots established in 1969 and 1970 were surveyed. Three 50-plant samples were examined for each determination. The results were as follows:

	% of Plants Affected				
	Lakeland			Wis. Syn H.	
Plants with	1968	1969	1970	1969	1970
virus	71	6	6	5	1
root borer	86	67	3	44	2

The effect of bean yellow mosaic virus on the productivity, in vitro digestibility, and nitrogen and chlorophyll concentrations was examined using susceptible red clover clones from two varieties. The virus had no effect on the digestibility of the plants, but it did cause an increase in nitrogen concentration and a decrease in chlorophyll concentration and forage yield. Digestibility estimates obtained on healthy plants were associated positively with nitrogen concentration (r=.50), leaflet-to-stem dry matter (r=.50), and leaf dry matter percentage (r=.35), and were associated negatively with shoot height (r=-.54) and stem dry matter percentage (r=-.30). Digestibility of the virus-infected plants was not associated with any agronomic or chemical attribute.

Digestible dry matter determinations were obtained on 38 red clover plants using the acid-pepsin and Tilley-Terry techniques. A significant positive correlation of 0.67 was observed between the two techniques. Acid-pepsin digestion was associated with nitrogen as determined by Kjeldahl (r=.71) and dye-binding (r-.65).

Experimental strains of red clover developed from the program at Wisconsin continued to outyield Lakeland. These strains have been selected for disease resistance (powdery mildew, northern anthracnose, and virus) and persistence.

State-wide tests provide data suggesting the superiority of several of these experimental strains. Seed of one strain, Wis. Syn H, was increased for further testing in 1971 and subsequent years.

Publications:

Smith, R. R. 1970. Application of male-sterility to forage legume breeding. Southern Forage Breeders Group Work Conference, July 14, 1970, Athens, Ga.

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GEORGIA

Dolichos lablab Evaluation and Breeding at Tifton, Georgia

Ian Forbes, Jr., Homer D. Wells, Norman A. Minton, and Warren G. Monson

Dolichos lablab L. has been one of the most promising "new" summer annual legumes tested in the forage legume species evaluation program at the Georgia Coastal Plain Experiment Station. A partial list of the species favorable qualities include: 1) high forage dry matter production (up to about 4 tons/A), 2) high protein content of the forage (av. approx. 18% for the season), 3) high digestibility of forage (av. approx. 67% for the season), 4) later fall growth and more frost tolerance than the warm-season grass species commonly grown (thus providing forage in our most critical forage-deficient period of the year.

Of the many accessions tested, the Australian cultivar 'Rongai', to which the above forage yield and quality measurements apply, has produced the most dry matter. However, like nearly all our accessions, Rongai will not produce a seed crop at Tifton before killing frost because it requires long nights to flower. This is a serious defect because this species has large seeds making them costly to ship from non-local seed-producing locations. This character also complicates any attempt at improvement through breeding at the Tifton latitude.